

**From:** [Stuber, Robyn](#)  
**To:** [Morris, Cris@Waterboards](#)  
**Cc:** [Denton, Debra](#)  
**Subject:** RE: PMSD Info. from the Federal Register WET Rule  
**Date:** Friday, March 06, 2015 12:14:00 PM  
**Attachments:** [2002 WET Rule -PMSD.pdf](#)  
[image001.png](#)  
[image003.png](#)  
[image004.png](#)

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See highlighted text.

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**From:** Morris, Cris@Waterboards [mailto:[Cris.Morris@waterboards.ca.gov](mailto:Cris.Morris@waterboards.ca.gov)]  
**Sent:** Thursday, March 05, 2015 11:52 AM  
**To:** Stuber, Robyn  
**Cc:** Denton, Debra  
**Subject:** FW: PMSD Info. from the Federal Register WET Rule  
**Importance:** High

Okay, I just talked to Sam and he is still not convinced that PMSD is not applicable to the TST results. When I pointed out that Table 6 below is only applicable for Sublethal Hypothesis Testing Endpoints Submitted under NPDES Permits, he replied that the TST is also a hypothesis test. I understand that it is a null hypothesis test, but I could not refute his claim that Table 6 did apply. Any guidance would be greatly appreciated.

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**From:** Morris, Cris@Waterboards  
**Sent:** Thursday, March 05, 2015 10:39 AM  
**To:** Unger, Samuel@Waterboards; Kuenzi, Nicole; Hung, David@Waterboards; Smith, Deborah@Waterboards  
**Cc:** Medina, Raul@Waterboards; Cuevas, Veronica; Erickson, Elizabeth@Waterboards; Webb, Steven [J.\[@Waterboards\]\(#\)](#)  
**Subject:** FW: PMSD Info. from the Federal Register WET Rule  
**Importance:** High

I have updated my concentration response “white paper” and the corresponding table. They are attached. The far right column in the table indicates the concerns and why each of the review steps are necessary. This should explain that PMSD is not applicable to the TST statistical approach. Veronica and I have also included some excerpts from the guidance below. If you are still not convinced, please let me know. I think it is very important that we not allow PMSD as a component of County San’s arguments so we have slide 30 rather than slide 29.

From Short term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, October 2002 (EPA 821-R-02-013). Note 10.2.8.2. Our required statistical method is the TST which does not require sublethal hypothesis testing endpoints. Thus the variability criteria (PMSD) is not required (nor is it applicable) for the TST. Table 6 below from the same document shows the acceptable range of PMSD and specifies that it is for “Sublethal Hypothesis Testing Endpoints Submitted under NPDES Permits”.

## 10.2.8 TEST VARIABILITY

10.2.8.1 The within-test variability of individual tests should be reviewed. Excessive within-test variability may invalidate a test result and warrant retesting. For evaluating within-test variability, reviewers should consult EPA guidance on upper and lower percent minimum significant difference (PMSD) bounds (USEPA, 2000b).

10.2.8.2 When NPDES permits require sublethal hypothesis testing endpoints from Methods 1000.0, 1002.0, or 1003.0 (e.g., growth or reproduction NOECs and LOECs), within-test variability must be reviewed and variability criteria must be applied as described in this section (10.2.8.2). When the methods are used for non-regulatory purposes, the variability criteria herein are recommended but are not required, and their use (or the use of alternative variability criteria) may depend upon the intended uses of the test results and the requirements of any applicable data quality objectives and quality assurance plan.

10.2.8.2.1 To measure test variability, calculate the percent minimum significant difference (PMSD) achieved in the test. The PMSD is the smallest percentage decrease in growth or reproduction from the control that could be determined as statistically significant in the test. The PMSD is calculated as 100 times the minimum significant difference (MSD) divided by the control mean. The equation and examples of MSD calculations are shown in Appendix C. PMSD may be calculated legitimately as a descriptive statistic for within-test variability, even when the hypothesis test is conducted using a non-parametric method. The PMSD bounds were based on a representative set of tests, including tests for which a non-parametric method was required for determining the NOEC or LOEC. The conduct of hypothesis testing to determine test results should follow the statistical flow charts provided for each method. That is, when test data fail to meet assumptions of normality or heterogeneity of variance, a non-parametric method (determined following the statistical flowchart for the method) should be used to calculate test results, but the PMSD may be calculated as described above (using parametric methods) to provide a measure of test variability.

TABLE 6. VARIABILITY CRITERIA (UPPER AND LOWER PMSD BOUNDS) FOR SUBLETHAL HYPOTHESIS TESTING ENDPOINTS SUBMITTED UNDER NPDES PERMITS.<sup>1</sup>

Test Method	Endpoint	Lower PMSD Bound	Upper PMSD Bound
Method 1000.0, Fathead Minnow Larval Survival and Growth Test	growth	12	30
Method 1002.0, <i>Ceriodaphnia dubia</i> Survival and Reproduction Test	reproduction	13	47
Method 1003.0, <i>Selenastrum capricornutum</i> Growth Test	growth	9.1	29

<sup>1</sup> Lower and upper PMSD bounds were determined from the 10<sup>th</sup> and 90<sup>th</sup> percentile, respectively, of PMSD data from EPA's WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b).

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**From:** Cuevas, Veronica@Waterboards  
**Sent:** Thursday, March 05, 2015 9:20 AM  
**To:** Morris, Cris@Waterboards  
**Subject:** PMSD Info. from the Federal Register WET Rule  
**Importance:** High

We refer to the WET Rule to determine what was the intent behind the use of the PMSD. The WET Rule is the Federal Register notice that was published by USEPA when they ratified approval of several test methods for measuring toxicity of effluents and receiving waters in 2002. This WET Rule is considered an update to the previously existing Chronic Toxicity Test Method for Freshwater.

It recognizes that variability criteria must be applied as a **test review step** (NOTE: not a Test Acceptability Criteria, but a test review criteria).

It identifies PMSD as the variability criteria that is used **when NPDES permits require NOEC** requirements, i.e. limits expressed as TUC. (1 TUC = 100 x 1/NOEC).

It elaborates that the PMSD should be compared to the upper bound and the lower bound.

clarify that receiving waters, synthetic waters, or synthetic waters adjusted to approximate receiving water characteristics may be used for dilution water, provided that the water meets the qualifications for an acceptable dilution water. EPA clarified in the method manuals that an acceptable dilution water is one which is appropriate for the objectives of the test; supports adequate performance of the test organisms with respect to survival, growth, reproduction, or other responses that may be measured in the test (*i.e.*, consistently meets test acceptability criteria for control responses); is consistent in quality; and does not contain contaminants that could produce toxicity. EPA also provided clarification on the use of dual controls. When using dual controls, the dilution water control should be used for determining the acceptability of the test and for comparisons with the tested effluent. If test acceptability criteria (*e.g.*, minimum survival, reproduction, or growth) are not met in the dilution water control, the test must be repeated on a newly collected sample. Comparisons between responses in the dilution water control and in the culture water control can be used to determine if the dilution water, which may be a receiving water, possesses ambient toxicity.

#### 6. Pathogen Interference

In today's action, EPA finalizes the proposed guidance on controlling pathogen interference in the Fathead Minnow Larval Survival and Growth Test with several modifications to address commenter concerns. Some commenters were concerned that the proposed guidance allowed the use of pathogen control techniques such as UV, chlorination, filtration, and antibiotics only after the recommended modified test design (fewer fish per cup) failed to control pathogen interference. Today's revisions clarify that EPA recommends pathogen control techniques that do not modify the sample, such as the modified test design technique, over ones that do. Upon approval by the regulatory authority, however, analysts also may use various sample sterilization techniques that modify the sample to control pathogen interference, provided that parallel testing of altered and unaltered samples further confirms the presence of pathogen interference and demonstrates successful pathogen control.

The manuals also now provide further explanation regarding the purpose for and required extent of pathogen source determination. Commenters were concerned that EPA was requiring

permittees to generate data that was irrelevant to correcting for pathogen test interference. This is not the case. Determining whether tests are adversely affected by pathogens in the effluent or pathogens in the receiving water used for test dilution is an important first step in selecting an appropriate pathogen control technique. If the source of interfering pathogens in the test is the receiving water used as the dilution water, then pathogen interference may be controlled by simply using an alternative dilution water. If the source of interfering pathogens in the test is the effluent, then pathogen control techniques are appropriate to control the interference. To further address the comments, EPA removed mention of pathogen source identification beyond determining whether the pathogen source was the effluent or dilution water. EPA also made several minor modifications in response to comments, including an acknowledgment that pathogen control techniques may not eliminate pathogens, but should minimize the adverse influence of pathogens so that test results are not confounded by mortality due to pathogens.

#### 7. EDTA in the *Selenastrum capricornutum* Growth Test

In the WET Interlaboratory Variability Study, EPA found that performance of the *Selenastrum capricornutum* Growth Test was much higher (lower interlaboratory variability and lower false positive rate) when the test was conducted with EDTA (ethylenediaminetetraacetic acid). Based on this finding, EPA proposed to recommend the use of EDTA in the *Selenastrum capricornutum* Growth Test. Several commenters expressed concern that EPA only recommended, rather than required, the use of EDTA. Commenters stated that this recommendation was not sufficient to ensure the acceptable performance of the method and encouraged EPA to require the use of EDTA. To address these comments, the *Selenastrum capricornutum* Growth Test now requires the addition of EDTA to nutrient stock solutions when conducting the *Selenastrum capricornutum* Growth Test and submitting data under NPDES permits. To address concerns that EDTA may interfere with (*i.e.*, mask) the toxicity of metals, the method continues to caution that the addition of EDTA may cause the *Selenastrum capricornutum* Growth Test to underestimate the toxicity of metals. EPA cautions regulatory authorities to consider this possibility when selecting test methods for

monitoring effluents that are suspected to contain metals. As recommended in EPA's Technical Support Document for Water Quality-Based Toxics Control (TSD) (USEPA, 1991), the most sensitive of at least three test species from different phyla should be used for monitoring the toxicity of effluents.

#### B. Additional Revisions to WET Test Methods

##### 1. Variability Criteria

Today's action incorporates mandatory variability criteria for five chronic test methods. EPA recommends the use of point estimation techniques over hypothesis testing approaches for calculating endpoints for effluent toxicity tests under the NPDES Permitting Program. However, to reduce the within-test variability and to increase statistical sensitivity when test endpoints are expressed using hypothesis testing rather than the preferred point estimation techniques, variability criteria must be applied as a test review step when NPDES permits require sublethal hypothesis testing endpoints (*i.e.*, no observed effect concentration (NOEC) or lowest observed effect concentration (LOEC)) and the effluent has been determined to have no toxicity at the permitted receiving water concentration. These variability criteria must be applied for the following methods: Fathead Minnow Larval Survival and Growth Test; Ceriodaphnia dubia Survival and Reproduction Test; *Selenastrum capricornutum* Growth Test; *Mysidopsis bahia* Survival, Growth, and Fecundity Test; and Inland Silverside Larval Survival and Growth Test. Within-test variability, measured as the percent minimum significant difference (PMSD), must be calculated and compared to upper bounds established for test PMSDs. Under this new requirement, tests conducted under NPDES permits that fail to meet the variability criteria (*i.e.*, PMSD upper bound) and show "no toxicity" at the permitted receiving water concentration (*i.e.*, no significant difference from the control at the receiving water concentration or above) are considered invalid and must be repeated on a newly collected sample. Lower bounds on the PMSD are also applied, such that test concentrations shall not be considered toxic (*i.e.*, significantly different from the control) if the relative difference from the control is less than the lower PMSD bound.

In the proposed rule, EPA solicited comment on the required use of upper and lower PMSD bounds in the calculation of NOEC and LOEC values.

Since the San Jose Creek permit, and others adopted recently no longer contain chronic toxicity limits expressed as NOEC or TUC, review of PSMD is not applicable.

With the limits expressed as Pass or Fail and % Effect, using the TST Welch's t-test to run the statistical analysis, there are other things that can be reviewed to look at variability, such as the coefficient of variation CV and the mean.

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